<Division of Biochemistry> Biofunctional Design-Chemistry

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- Biofunctional Design-Chemistry -

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Visitors

Prof MEZO, Gabor — Hungarian Academy of Sciences, Hungary, 24 - 26 October 2005
Prof FUDECZ, Ferenc — Hungarian Academy of Sciences, Hungary, 25 - 26 October 2005
Mr BANOCZI, Zoltan — Hungarian Academy of Sciences, Hungary, 11 July - 13 August 2005

Scope of Research

The ultimate goal of our research is the regulation of cellular functions by designed peptides and proteins. Current research subjects include (1) development of novel intracellular delivery systems aiming at elucidation and control of cellular functions using designed membrane permeable peptide vectors, (2) elucidation of the DNA binding and recognition modes of C2H2-type zinc finger proteins and design of artificial transcription factors with various DNA binding specificities, and (3) design of stimulation-responsible artificial peptides and proteins.

Research Activities (Year 2005)

Presentations

"Cell-penetrating Peptides and Applications" Futaki S, Wenner-Gren Foundations International Symposium, Stockholm, Sweden, 19 May.

"Control of Peptide Structure and Function by Fe(III)-Induced Helix Destabilization" Futaki S, Kiwada T, Sugiura Y, 19th American Peptide Symposium, San Diego, USA, 18 - 23 June.

"Effect of Counteranions and Membrane Potential on the Cellular Uptake of Arginine-rich Peptides" Takeuchi T, Nakase I, Sugiura Y, Sakai N, Matile S, Futaki S, 19th American Peptide Symposium, San Diego, USA, 18 - 23 June.

"Artificial 6-zinc Finger Peptides with Various Linkers" Imanishi M, Sugiura Y, Metalloprotein and Protein Design Conference, Chicago, USA, 30 July.

"Counteranion-Mediated Delivery of Arginine-Rich Peptides into Living Cells" Takeuchi T, Kosuge M, Tadokoro A, Sugiura Y, Nishi M, Kawata M, Sakai N, Matile S, Futaki S, 42th Japanese Peptide Symposium, Suita, 28 October.

"The Effect of Octaarginine on the Translocation of Daunomycin-branched Polypeptide Conjugates" Banoczi Z, Remenyi J, Takeuchi T, Futaki S, Fudeczl F, 42th Japanese Peptide Symposium, Suita, 28 October.

"Peptide Vectors for Intracellular Delivery" Futaki S, 2nd Annual Symposium Japanese-German Frontiers of Sciences, Zushi, 4 November.

Grants

Futaki S, Development of Intracellular Targeting Vectors and the Real-time Observation of the Intracellular Delivery, Grant-in-Aid for Scientific Research (B), 1 April 2005 - 31 March 2008.

Futaki S, Functional Design of Cell-targeting Peptides, PRESTO Program, Japan Science and Technology Agency, 1 November 2002 - 31 October 2005.

Imanishi M, Screening and Evaluation of Novel Clock-related Proteins Using Zinc-finger Technology, PRESTO Program, Japan Science and Technology Agency, 1 October 2005 - 31 March 2009.

A pH-sensitive Fusogenic Peptide to Improve the Transfection Efficiency of Cationic Liposomes

One of the most important steps in intracellular gene-delivery using cationic liposomes is the endosomal escape of the plasmid/liposome complexes to cytosol. The addition of a pH-sensitive fusogenic peptide such as GALA (WEAALAEALAEALAEHLAEALAEALEALAA) may accelerate this step to enhance the expression of the desired proteins. Using representative commercially available cationic liposomes (Lipofectin, Lipofectamine, and Lipofectamine 2000), the effects of GALA on the transfection efficiency were studied by luciferase assay and confocal microscopic observations. A concentration-dependent increase in the transfection efficiency was observed, and addition of 0.1 mM GALA to the plasmid/liposome complex significantly increased the transfection efficiency, especially in the case of Lipofectin. While maintaining a high transfection efficiency, reduction in the liposomal dosage was attained by employing GALA. Although the transfection efficiency was higher in the presence of GALA, a lower amount of the plasmid DNA was taken up by the cells. Confocal microscopic observations of the rhodamine-labeled plasmid show little difference in the cellular localization among cells incubated in the presence or absence of GALA, suggesting that a sublte increase in GALA-induced release of the pasmid to the cytosol may yield a considerable difference in the transfection efficiency. Based on these findings, novel concepts and systems for intracellular gene delivery should be established using pH-sensitive fusogenic peptides.

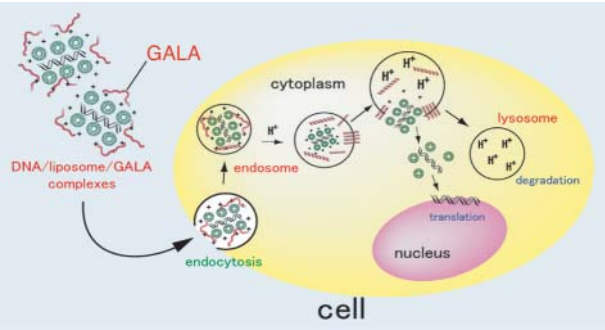

Figure 1. GALA accelerates the endosomal escape of DNA/liposome complexes.

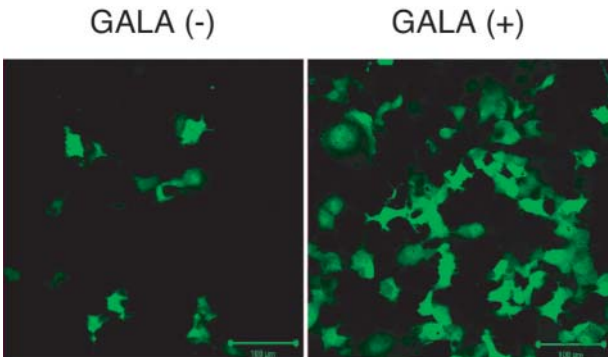

Figure 2. GALA enhances the expression of enhanced green fluorescent protein (EGFP) in COS-7 cells.

Selective Binding to the Discontinuous DNA Sequences by an Artificial Zinc Finger Peptide with Polyarginine Linker

Artificial DNA binding peptides recognizing separated sequences would expand the varieties of the DNA sequences as target sites for transcriptional control. We created a 6-zinc finger peptide, Sp1ZF6(Arg)8, by connecting two DNA binding domains of transcription factor Sp1 with a cationic and bulky polyarginine linker. The DNA binding properties to continuous and discontinuous target sequences were examined and compared to those of Sp1ZF6(Gly)10, which contains a flexible and neutral polyglycine linker. Sp1ZF6(Arg)8 was demonstrated to have an obvious DNA binding preference to discontinuous target sequences, while Sp1ZF6(Gly)10 bound to the both. Footprinting analyses showed that Sp1ZF6(Arg)8 specifically binds to the discontinuous binding sites but not to the continuous ones. Bulky and cationic polyarginine linker may inhibit the suitable binding of Sp1ZF6(Arg)8 to the continuous target sequence, resulting in strong preference to the discontinuous ones. The results provide helpful information for linker design of future zinc finger peptides targeting various states of DNA in addition to desired sequences as gene expression regulators.

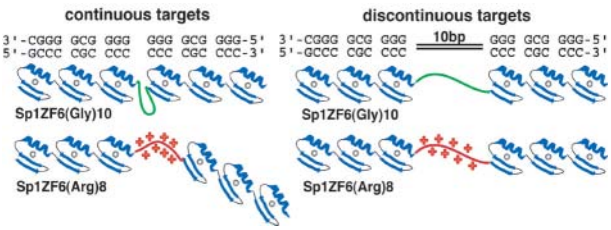

Figure 3. Schematic representation of selective binding of Sp1ZF6(Arg)8 to the discontinuous DNA sequences.